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SLC35A2-related congenital disorder of glycosylation: defining the phenotype

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Cover Letter

Dear Professor Zuberi,

Many thanks for your consideration of our manuscript. We have addressed the reviewers' comments in detail below. Changes to the manuscript have been highlighted in green. We hope that our revisions meet with your approval.

Comments from the editors and reviewers:
-**Reviewer 1**

-
Yates et al. present a series of five female patients with pathogenic variants in the SLC35A2 gene which is associated with Congenital Disorders of Glycosylation (CDG) type IIm. They have also sought to review the existing literature in order to define the phenotype of this condition more clearly.
The data they provide in support of the pathogenicity of these variants is strong, particularly since all have arise de novo. They have also gathered a reasonable amount of clinical detail in each case. The novel aspects that this paper provides on SLC35A2 are that they report cerebral visual impairment as a phenotypic feature, and they report Tetralogy of Fallot in one case.
I feel that there has been a slight missed opportunity here to provide the reader with a better grasp of this rare condition. Too much manuscript space has been devoted to the narrative of the case histories (details of which are completely replicated in table 1) whilst the phenotypic detail provided on the 14 published cases is a little weak (for example, we are only told if they have seizures or not, not what type of seizure nor the age of presentation).
Thank you for this comment. The table describing all published cases has been updated with age of seizure onset and types of seizures.
Further detail has been added in the discussion in respect to the seizure phenotype and MRI brain findings, including previously published cases.

An overall analysis of the phenotypic spectrum (taking new and published cases into account) is lacking.
Many thanks. A section has been added to the discussion to summarise the cardinal features of the published cases.

Given the lack of precision of detail on medication response I do not think the title "treatment strategies" is appropriate.
Thank you. The title has now been altered.

The methods section of this manuscript is unsuitably short and vague.
Many thanks – the methods section has been updated with additional detail regarding sequencing methodology. The methods are described in further detail in the supplementary material.

Re; Title:

Can you call this “SLC35A2-related epileptic encephalopathy: patient series” when one of the cases you report did not have epilepsy? Can you call this “treatment strategies” when you provide no substantive evidence to support any given treatment approach.
Many thanks for this. The title has been altered.

Re: Methods:
Reading through the supplementary material and looking at the affiliations of the authors it is apparent that these patients have been recruited and had genetic testing in a variety of centres. It is not clear in the methods therefore how this cohort has been put together. It would be helpful if the authors could explain this in more detail in the methods – for example was a gene matching website used?
Thank you. Details have been added to the methods section regarding the cohort and how it was formed.

Also it should be explained on what basis patients at each centre were selected for genetic testing since this will affect the ascertainment of the cases and the phenotype described. For example if one centre was only doing WES on patients with infantile spasms this would result in a potential bias which ought to be disclosed.
NEED TO DOUBLE CHECK WITH AUTHORS

Re: Results:
Most of the details within the patient reports section of the results are duplicated in table 1. The patient reports are largely in keeping with the previously published cases (apart from the new features of visual impairment and Tetralogy of Fallot) so this section does not provide any novel insights. I think the manuscript would be stronger if the results section took a more analytical approach to the epilepsy phenotypes of the new and published cases. If they did this then a better title would perhaps be “SLC35A2: Defining the epilepsy phenotype.” (see Title comment above)
Thank you. The results section now includes a more in-depth analysis of the epilepsy phenotype.

The precision of detail on medication response is inadequate. What does “partial response” (case 1, case 2) mean and who’s assessment was this? A more structures approach to assessment and reporting of medication response is needed.
Thank you - we define partial response as a decrease in seizure frequency and/or duration without complete control of epilepsy. This was assessed by the individual clinicians looking after each patient, which was a Clinical Geneticist and Paediatric Neurologist in most cases.

Minor:
Consistency with hyphenation see “to-date” or “to date”
Thank you, this has been updated.

With X-inactivation say which allele the skewing is in favour of

Thank you – the skewing is 100% therefore this is in favour of the pathogenic allele.

Consider use of most up to date ILAE terminology. See Scheffer 2017, Epilepsia
- - “Developmental and epileptic encephalopathy” not “epileptic encephalopathy”
- - “Drug-resistant epilepsy” not “refractory”
Thank you, this has been altered accordingly.

Do these patients truly have West syndrome? Or do you think that the definition of West syndrome depends on developmental plateau or regression?
Thank you – in the manuscript, we have defined West syndrome as the triad of infantile spasms, hypsarrhythmia and developmental delay rather than plateau/regression.

It would be good to have data on any developmental assessments prior to presentation with epileptic spasms.
Thank you for this comment. Unfortunately, as most patients present with early-onset seizures, we would have to assume that developmental delay presented before epilepsy.

It is difficult to make any comment on treatment response in this specific group of patients. The treatments reported as successful here are the ones that most typically used to treat infantile spasms. The authors should acknowledge this as a limitation.
Thank you, the treatment section in the discussion has been altered to reflect the standard treatments being used and lack of specific therapies in this group of patients.

Discussion would benefit from a broader discussion of the relationship between CDGs and severe early onset epilepsy. It would seem that this CDG is quite unique in that nearly all known cases have a severe early onset epilepsy. I think this is worth mentioning.
Thank you, a mention of this has been added to the discussion.

At the bottom of paragraph 2 page 12 there is a line “In the literature, these have been reported at approximately equal frequency.” I think this is with reference to missense and nonsense variants being observed with equal frequency, but this is not clear. There are also frameshift variants observed in the literature which you have not mentioned.
Thank you. This has been revised to state that missense and truncating variants have been reported with equal frequency.

Would you recommend transferrin isoelectric focussing as a diagnostic test when a female presents with infantile spasms? This series would suggest that this does not usually happen.
Thank you, a sentence has been added to recommend this as a diagnostic test, emphasising the need to perform this before three years of age.

Tables:
If you want to include reference to treatment response in the paper please include AEDs used and what treatment response was seen (as precisely as possible) in table 1.
Thank you, however, we do not have enough data to make firm conclusions on this.

Please include details of MRI abnormalities in table 1
Many thanks, these details have been added to the table.

Please include head circumference measurements and centiles in table 1, if available.
Thank you, the centiles for head circumference have been added.

Please include severity of cognitive impairment and details of seizure type (at presentation and evolution) in table 2.

Thank you, the severity of developmental delay/intellectual disability has been included in table 2. Further details of seizures have also been added.

In table 2 please spell de novo fully rather than putting “dn”.
Thank you, this has been altered.

-**Reviewer 2**

-
Yates and colleagues describe in detail the clinical phenotypes and treatment response of five patients with de novo disease causing variants in SLC35A2 and then summarise clinical details of published cases. The paper is well written and represents a significant addition to current literature as no similar detailed review exists.
I would recommend the following changes and corrections;
1. In the methods section, the authors should include brief details about how the patients were ascertained. This will allow the reader to understand any effect of ascertainment bias on reported phenotypic features.
NEED TO CHECK WITH AUTHORS RE ASCERTAINMENT AS ABOVE

2. Carbamazapine should be Carbamazepine
Thank you, this has been altered.

3. For consistency the authors should include brief details of the skeletal anomalies including coxa valga in the main case report for patient 1 as well as in the supplementary information.
Thank you, this information has been added to the case report.

4. The authors state that ‘The X-rays of the pelvis in both patients 1 and 2 showed bilateral coxa valga with high migration index on both sides, suggesting this may be a specific finding.’ Therefore radiographs illustrating this from patient 1 as well should be included if possible.

Thank you, unfortunately these radiographs were not available for this revision.

5. Cortical visual impairment is due to CNS dysfunction and is not strictly an ophthalmic abnormality. Visual defect may be a better term.
Thank you, 'ophthalmic abnormality' has been altered to 'visual defect' in both the text and table 2.

6. Table 1 does not need to include a full text description of patient dysmorphology. Instead this should be moved to the supplementary case reports.
Thank you, the description of dysmorphology has been moved to case reports.

7. In table 1 OFC centiles should be included in the microcephaly line
Thank you, microcephaly has been replaced by OFC centile.

8. Genomic coordinates for all variants should be included in table 1.
Thank you for this comment, but as details of the transcript and genome build are included we do not feel the genomic coordinates are necessary. Details of the transcript number have been added to the table legend.

9. In table 2 in the lines for MRI, skeletal and ophthalmic abnormality it would be better to include a couple of words or an abbreviation summarising the abnormality rather than Y and N with the description being in the legend. The reader could then see the spectrum of abnormalities more easily.
Thank you. The table has been altered to include abbreviations summarising the abnormalities found.

10. 'Visual defect' or 'Visual impairment' is preferable to 'Ophthalmic abnormality' because cortical visual impairment is not strictly an ophthalmic abnormality.
Thank you, 'ophthalmic abnormality' has been altered to 'visual defect'

11. 'Low set hair' should be 'low anterior hairline'.
Thank you. This has been altered in the supplementary data.

12. Figure 1 legend – 'a protruding tongue note.' should be corrected.
Thank you, this has been corrected.

13. Figure 1 – the photos do not appear to confirm mild 2-3 toe syndactyly in patient 3.
Thank you, reference to this has been removed.

14. Figure 1 – patient 3. It isn't clear what 'slightly overhanging columella' means – suggest omitting this.
Thank you, this has been omitted.

15. 'None of the four patients reported here….'. The authors report describes five patients.

Thank you, this has been corrected to ‘five patients’.

16. The authors state that ‘common features include short stature…..coxa valga.’ None of these features appear to be particularly common so it would be better just to state that ‘Skeletal features included…...’
Thank you, this has been altered to ‘Features include…’

Yours sincerely,

Dr Michael Yates MBChB MSc MRCP

Abstract

We aim to further delineate the phenotype associated with pathogenic variants in the SLC35A2 gene, and review all published literature to-date. This gene is located on the X chromosome and encodes a UDP-galactose transporter. Pathogenic variants in SLC35A2 cause a congenital disorder of glycosylation. The condition is rare, and less than twenty patients have been reported to-date. The phenotype is complex and has not been fully defined. Here, we present a series of five patients with de novo pathogenic variants in SLC35A2. The patients' phenotype includes developmental and epileptic encephalopathy with hypsarrhythmia, facial dysmorphism, severe intellectual disability, skeletal abnormalities, congenital cardiac disease and cortical visual impairment.

Developmental and epileptic encephalopathy with hypsarrhythmia is present in most patients with SLC35A2 variants, and is drug-resistant in the majority of cases. Adrenocorticotropic hormone therapy may achieve partial or complete remission of seizures, but the effect is usually temporary. Isoelectric focusing of transferrins may be normal after infancy, therefore a congenital disorder of glycosylation should still be considered as a diagnosis in the presence of a suggestive phenotype. We also provide evidence that cortical visual impairment is part of the phenotypic spectrum.

Running title: SLC35A2: defining the phenotype

Keywords: Developmental and epileptic encephalopathy, Congenital disorders of Glycosylation, Intellectual Disability, SLC35A2

Introduction

Over 100 glycosylation-related human genetic disorders have been reported in the literature. These result from disruption of the normal N-glycosylation pathway which involves adding complex sugar chains (glycans) to proteins[1]. Congenital disorders of glycosylation (CDG) can result in a complex phenotype, including intellectual disability and seizures[2-3]. Variants in the SLC35A2 gene (MIM #300896), on the X chromosome, coding for the only known transporter of UDP-galactose to the Golgi apparatus, have been found to result in a congenital disorder of glycosylation[4]. To the authors' knowledge, less than twenty patients with variants in this gene have been reported in detail to-date[3-10]. The associated phenotype includes developmental and epileptic encephalopathy (EE), severe intellectual disability (ID), hypotonia, dysmorphic features, and shortening of the distal limbs.

Here, we present five new patients with de novo pathogenic variants in SLC35A2, who have EE with hypsarrhythmia, severe ID, skeletal abnormalities and cortical visual impairment. We further define the phenotypic spectrum, in particular the epilepsy phenotype. With increasing use of next generation sequencing in patients with epilepsy, it is likely that more patients will be identified with this phenotype and hence, it is important to better understand the disease course and potential treatment options in this group of disorders.

Patient Reports

Our cohort consists of five female patients born to non-consanguineous White European parents (Fig.1). All patients had normal chromosomal microarray analysis (Patient 1 was found to have a paternally inherited 320 kb deletion at 6q26, classified as likely benign). All were found to have heterozygous de novo variants in SLC35A2 with no additional variants. Patient 4 was also found to have a skewed X chromosome inactivation profile (100:0). Three patients had normal isoelectric focusing of transferrins, performed between the ages of 2 and 8 years. In addition to the reports here, Table 1 provides further clinical information. Further details are included in the supplementary material.

Patient 1

This 3-year old patient developed infantile spasms at 6-weeks of age. EEG (electroencephalogram) demonstrated hypsarrhythmia. Magnetic Resonance Imaging (MRI) of the brain at 7-months of age demonstrated asymmetry of the lateral ventricles and a thin corpus callosum (Supp Fig. 1). She had a partial response to Vigabatrin and Prednisolone. Her seizures subsequently evolved to myoclonic and tonic seizures in addition to ongoing spasms with clusters. These were resistant to several different treatment regimens including combinations of Vigabatrin, steroids, Sodium Valproate, Levetiracetam, and Topiramate. Seizure control improved on classical ketogenic diet therapy at 12-months of age, together with cessation of all anti-epileptic medications. A course of ACTH (adrenocorticotropic hormone) therapy resulted in a definite,

but short-lived, improvement in seizure control. Response to a second course of ACTH was less effective.

She had significant developmental impairment. Tetralogy of Fallot was noted on antenatal ultrasound (USS), which subsequently required surgery. On assessment at the age of 3-years, she was noted to be dysmorphic and had right lower limb shortening. Radiological review of a skeletal survey demonstrated thoracic scoliosis, bilateral coxa valga and generalised osteopenia. There were unusual defects involving the right proximal and distal tibia, with the defect in the proximal tibia being larger. Overall, the right tibia was shorter than the left. Possible similar early changes were seen in the left distal tibia (Fig. 2). The significance of these radiological findings remains uncertain.

She also has severe cortical visual impairment, with no response to formal visual acuity assessment but some brief fixation. Electroretinogram (ERG) confirmed no evidence of diffuse retinal dysfunction.

Patient 2

This 8-year old patient developed infantile spasms at the age of 5-months. EEG showed hypsarrhythmia and an MRI brain scan was normal. Initial treatment was with Prednisolone and subsequently Vigabatrin with partial response. Over the course of the next few years she developed drop attacks, tonic spasms, and changes in visual attention associated with an unusual cry. Her seizures were resistant to ketogenic diet, as well as combinations of Lamotrigine, Zonisamide, Nitrazepam, Rufinamide, and Topiramate. Repeat

EEG at the age of 4-years and 3-months showed hypsarrythmia. Repeat MRI brain imaging at the ages of 18 and 31 months showed significantly delayed cerebral white matter myelination. This normalised by age 4-years, with some residual hyperintensity posterior to the trigones of the lateral ventricles. The hippocampi appeared small and malrotated (Supp Fig. 2).

She had severe developmental delay. On assessment at the age of 8-years she had severe ID and was able to walk a few steps with support. She was dysmorphic. A pelvis x-ray showed bilateral coxa valga with partial hip subluxation (Fig.2).

Patient 3

This 8-year old patient developed infantile spasms at 6-months of age. EEG demonstrated hypsarrhythmia. Her seizures responded to Vigabatrin in combination with ACTH. She was then treated with Valproate monotherapy. From the age of 3 ½ years, medication was withdrawn and she remained seizure-free. Neonatal nystagmus was noted, which ceased as seizure control improved. She had myopia, astigmatism, intermittent exotropia of the right eye and amblyopia ex anopsia. ERG performed at 14-months of age was normal. MRI brain and echocardiogram at the age of 8-months was normal.

On assessment at the age of 3-years and 4-months, she had severe hypotonia and severe global developmental delay. She was dysmorphic. At the age of 8-years, she has severe ID with no spoken language. She can walk with support if helped to a standing position.

Patient 4

This 3-year old patient developed infantile spasms at 6-weeks of age and EEG demonstrated hypsarrhythmia. Her spasms were partially controlled with a combination of Vigabatrin, steroids and ACTH therapy. She was treated unsuccessfully with Phenobarbital, Valproate, Levetiracetam and Topiramate. Her spasms ceased with a combination of Vigabatrin, Nitrazepam and ketogenic diet at 2-years and 3-months of age. MRI brain scan at two months was normal. A second MRI brain at 2 ½ years of age demonstrated thinning of the corpus callosum with delayed myelination and slight enlargement of the lateral ventricles. On assessment at 3-years and 4-months of age, she had global hypotonia. She could sit and stand with help. She had no spoken language. She had severe ID. She was dysmorphic. There were no ophthalmic abnormalities.

Patient 5

This 10-month old patient developed eye rolling and chewing episodes at the age of four months, as well as unilateral myoclonic jerks. There was one episode of opisthotonus. Focal epileptic activity was noted on EEG at the age of five months, however this did not correlate with clinical episodes. Carbamazepine and then Levetiracetam monotherapy was trialled, but did not have a significant effect and was discontinued. MRI brain was normal.

On assessment at the age of 10-months, she had moderate to severe developmental delay. She had hypotonia, and was dysmorphic. There were no ophthalmic abnormalities.

Methods

Patients 1,2 and 4 were ascertained through routine referral to their local Clinical Genetics service, with a referral indication of drug-resistant developmental and epileptic encephalopathy. The cohort was formed through personal communication (Patient 2), interrogation of the DECIPHER database[11] (Patient 3) and through the GeneMatcher website[12] (Patients 4 and 5). Genomic DNA was extracted from peripheral blood leukocytes from patients using standard procedures. All patients underwent chromosomal microarray-analysis.

For patients 1 & 2, sequencing of 72 genes associated with early infantile epileptic encephalopathy was carried out using targeted whole exome sequencing (WES) (Agilent SureSelect and MiSeq). Trio-based whole genome sequencing (WGS) was carried out for patient 3 using the Illumina HiSeqX system (Illumina). For patient 4, sequencing of a 93 gene panel for monogenic epileptic disorders was performed on a NextSeq500 (Illumina). WES was performed for patient 5 using NovaSeq 6000 Agilent SureSelect All Human Exon v6. All data was analysed against GRCh37. All variants reported are according to the NM_005660.2 transcript. Variants were verified by Sanger sequencing using standard protocols. Methods are described in further detail in the supplementary material.

Discussion

We present here a series of five patients with de novo pathogenic variants in SLC35A2 and phenotypic features including epilepsy with hypsarrhythmia, severe ID, non-specific dysmorphism, visual and skeletal abnormalities.

Comparison of our cohort with those previously reported[3-10] allows us to define the phenotype associated with SLC35A2, particularly in relation to epilepsy, which affects 16/18 (89%) patients (Table 2). Seizures start in early infancy, with 8/11 (73%) of those reported developing before the age of 3-months and all before 6-months. The presentation was with infantile spasms in 8/10 (80%). Two patients presented with tonic seizures and partial motor seizures respectively. The phenotype was reported, in six patients, to progress to a combination of tonic seizures plus spasms. Two patients were also reported to have myoclonic seizures. Hypsarrhythmia is common (13/16; 81%). Given that all affected patients have developmental delay, this means most meet the criteria for West syndrome (triad of infantile spasms, hypsarrhythmia and developmental delay). Epilepsy in this group is usually resistant to treatment with 64% of patients having ongoing seizures.

There does not appear to be a definite pattern to response in treatment, but our experience suggests that partial and/or temporary seizure control may be achieved with either Vigabatrin +/- steroids, the ketogenic diet or ACTH. Four previously reported patients have had a temporary response to ACTH, with a further patient achieving seizure control with this therapy[3,6-7]. As these are

standard treatments for infantile spasms, further work is needed to determine optimal epilepsy medication in this group of patients.

All patients reported have severe ID. None of the five patients reported here had developed spoken language. Non-specific dysmorphic features are present in 16/17 (94%). Hypotonia (12/18; 67%) and microcephaly (6/13; 46%) are common. Fifteen out of 18 (83%) patients have abnormalities on brain MRI. The most common findings are thinning of the corpus callosum (8/18; 44%), cerebral and/or cerebellar atrophy (8/18; 44%) and delayed myelination (6/18; 33%). One patient in our cohort had a normal MRI brain scan at the age of 8-months. Interestingly, however, two previously reported patients[3] initially had normal MRI-brain scans in infancy. Subsequent imaging at the ages of 4 and 8 years respectively was abnormal. Therefore, it is possible that SLC35A2-associated brain abnormalities develop over time, and this may well prove to be the case in our patient with an early normal MRI brain scan.

Visual defects are present in 11/16 (69%) individuals. Of note, one other patient has been previously reported with cortical visual impairment[5], although the patient of Dörre et al.[6] had severe visual impairment of unspecified cause. Therefore, our report provides further evidence that this specific visual abnormality is part of the phenotypic spectrum associated with SLC35A2 pathogenic variants. However, it is also possible that in view of EE, visual impairment may be a part of the phenotype rather than a specific issue.

Skeletal abnormalities are found in 9/14 (64%) patients. Features include short stature, non-specific limb shortening, scoliosis and bilateral coxa valga. The X-rays of the pelvis in both patients 1 and 2 showed bilateral coxa valga with high migration index on both sides, suggesting this may be a specific finding. Further imaging of patients with SLC35A2 is required to ascertain the skeletal findings in this group.

Our cohort includes one patient with Tetralogy of Fallot. This has not been reported in association with SLC35A2 and may represent a novel feature. However, this cardiac abnormality can occur in isolation and further reports are required to determine if congenital cardiac disease is part of the SLC35A2 phenotypic spectrum.

Transferrin isoelectric focusing was normal for those tested in our cohort at ages 3-years 4-months and 2-years 6-months respectively (Patients 3 & 4). The majority of reported patients, including our cohort, had normal transferrin profiles after the age of 2-years (Table 2). Ng et al.[4] demonstrated galactosylation-deficient transferrin profiles in their patient series between the ages of 5-7 months, with normalisation after the age of 3 years. Therefore, there is likely to be an age-related time period for transferrin testing in relation to SLC35A2-related CDG, emphasising the importance of early diagnosis. Ng et al.[4] hypothesised this is related to selection against hepatocytes with abnormal SLC35A2 during early development; however, this has not been conclusively demonstrated. Our data suggest that isoelectric focusing of transferrins is a useful diagnostic test in females presenting with early onset

infantile spasms. This analysis should be performed before three years of age if feasible.

SLC35A2 is located on the X chromosome; all of our patients are females, in keeping with majority of patients reported thus far. Ng et al.[4] reported two male patients who were mosaic for SLC35A2 pathogenic variants. It is likely that the presence of a functional allele is required for survival. There have been two reports of maternal inheritance in the literature[8,10]; otherwise all the pathogenic variants reported are de novo. Our cohort includes three novel missense variants and one novel nonsense variant. Overall, including our cohort, missense and truncating variants have been reported in the literature at approximately equal frequency.

In summary, we present a series of five patients with pathogenic SLC35A2 variants and review the features of patients previously reported, with particular focus on the drug-resistant epilepsy phenotype and MRI brain findings. This allows us to further define the phenotype of this rare disorder. Early onset epilepsy presenting with infantile spasms and hypsarrhythmia predominates, in association with severe developmental delay and ID. Interestingly, severe early-onset epilepsy is unusual in CDGs in general. This appears to be a feature unique to SLC35A2-related CDG.

Additional common features include non-specific dysmorphism, microcephaly, hypotonia and MRI-brain abnormalities (e.g. thinning of the corpus callosum

and cerebral atrophy). Visual and skeletal defects may be present, but further data is required to determine how specific these are in relation to SLC35A2. In this respect, we provide evidence that cortical visual impairment is part of the phenotypic spectrum. Additionally, congenital cardiac disease may be part of the extended phenotype. Further patient series of this nature are required to understand the natural history and course of the seizure phenotype in patients with rare genotypes, such as those with pathogenic variants in SLC35A2.

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Figure legends

Figure 1. Patients 1 (a,d,g,h), 2 (b,e) and 3 (c,f,i,j) at ages 11-months, 8-years and 11-months, and two-years respectively. **Patient 1:** dysmorphic features include brachycephaly, hypertelorism, and low-set ears. Note 2-3 toe syndactyly (R foot) and proximally placed 2nd toe (L foot). **Patient 2:** dysmorphic features include high-set eyebrows, long, narrow palpebral fissures, mid-face hypoplasia with depressed nasal bridge, open mouth with full, tented upper lip and a protruding tongue. **Patient 3:** dysmorphic features include elongated palpebral fissures, large blue irises; eversion of the lower lids; long and prominent eyelashes; broad eyebrows; short nose and hypoplastic alae nasi; short, grooved philtrum; tented upper lip and posteriorly-rotated ears. Also note tapering fingers and broad great toes.

Figure 2. Patient 1 (a-b): radiographs at 11-months of age demonstrating non-specific defects involving the right proximal and distal tibia, with the proximal tibia being larger. Patient 2 (c): radiograph of pelvis demonstrates bilateral coxa valga with partial hip subluxation.

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Gender	F	F	F	F	F
Age at evaluation (yr;mo)	3	8;11	8;0	3;4	0;10
SLC35A2 variant (predicted protein change)	c.889A>G (p.Lys297Glu)	c.327T>G (p.Tyr106*)	c.195C>A (p.Phe65Leu)	c.515T>C (p.Leu172Pro)	c.923C>T (p.Ser308Phe)
ACMG pathogenicity criteria	PS2, PM2 and PP3 (Likely pathogenic)	PVS1,PS2 (Pathogenic)	PS2, PM2, PP3, PP4 (Likely pathogenic)	PS2, PM2 and PP3 (Likely pathogenic)	PS2, PM2, PP2, PP3 (Likely pathogenic)
Family history	Cousin X-linked agammaglobulinaemia	Brother single febrile seizure	Unremarkable	Unremarkable	Unremarkable
Antenatal findings	Tetralogy of Fallot	Nil	Nil	Nuchal thickening	Nil
Gestational age at birth (weeks)	40	34	43	40	38
Birth weight	50th centile	25-50th centile	25th centile	40th centile	75th-91st centile
Admission to NICU	Yes – Tetralogy of Fallot	Yes - Weight loss, jaundice, skin ulcers	No	No	Yes - CPAP
Dev. delay	Yes	Yes	Yes	Yes	Yes
ID	n/a	Severe	Severe	Severe	n/a
Age of seizure onset	6 weeks	5 months	6 months	6 weeks	Possible at age 4 months
Initial seizure type	Infantile spasms	Infantile spasms	Infantile spasms	Infantile spasms	Eye rolling/chewing episodes
Evolving seizure phenotype	Myoclonic & tonic seizures; and ongoing spasms with clusters	Drop attacks, tonic spasms, change in visual attention	n/a	n/a	n/a
EEG	Hypsarrhythmia	Hypsarrhythmia	Hypsarrhythmia	Hypsarrhythmia	Focalised epileptic activity
AED response	Partial	Pharmacoresistant	Seizure free, off medication	Seizure free on Vigabatrin, Nitrazepam and ketogenic diet	AED stopped with no clinical change

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Dysmorphic	Yes	Yes	Yes	Yes	Yes
Hypotonia	No	No	Yes	Yes	Yes
Head circumference centile (age)	9th (11mo)	<<0.4th (9yr)	98th-99.6th (birth)	40th (3y 4mo)	50th (10mo)
Visual defect	Severe cortical visual impairment	No	Myopia, astigmatism, intermittent exotropia of the right eye and amblyopia ex anopsia	No	Strabismus
Skeletal abnormality	Thoracic scoliosis, bilateral coxa valga and generalised osteopenia. Unusual defects involving the right proximal and distal tibia, with right tibia shorter than the left. Possible similar early changes in the left distal tibia	Bilateral coxa valga with partial hip subluxation	Joint hypermobility, genu valga, asymmetric chest deformity	Camptodactyly of the 2nd right finger	n/a
MRI abnormality (age)	Asymmetry of the lateral ventricles and a thin corpus callosum (7 mo)	Normal (5mo). Delayed cerebral white matter myelination (18 & 31mo). Normalised with some residual hyperintensity posterior to the trigones of the lateral ventricles (4yr). Hippocampi small and malrotated	No	Normal (2mo). Thinning of the corpus callosum with delayed myelination and slight enlargement of the lateral ventricles (2.5yr).	No
Isf Tf (age -yr;mo)	nd	normal (8;11)	normal (3;4)	normal (2;6)	nd

Table 1. Features of patients in this cohort. Isf Tf isoelectric focusing of transferrins; n/a not applicable; nd not done; AED antiepileptic medication. All variants are according to the NM_005660.2 transcript.

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Ng et al. (Patient 1)	Ng et al. (Patient 2)	Ng et al. (Patient 3)	Kodera et al. (Patient 1)	Kodera et al. (Patient 2)	Kodera et al. (Patient 3)	Bosch et al.	Kimizu et al.	Dorre et al.	Lopes et al.	EuroEPINOMICS et al (Patient 1)	EuroEPINOMICS et al (Patient 2)	Evers et al.	Total (reported)
Gender	F	F	F	F	F	M	F	M	F	F	F	F	F	F	M	F	F	F	-
Age (yr;mo)	3	8;11	8;0	3;4	0;10	3	3	6	8;10	12;8	10;5	23	2	5	8	3	13	2	-
Inher.	de novo	de novo	de novo	de novo	de novo	de novo	de novo	de novo	de novo	de novo	de novo	de novo	de novo	de novo	mat	de novo	de novo	mat	-
SLC35A2 variant	c.889A>G	c.327T>G	c.195C>A	c.515T>C	c.923C>T	c.15_91+48delinsA	c.3G>A	c.991G>A	c.433_434del	c.972del	c.638C>T	c.800A>G	c.950delG	c.797G>T	c.772G>A	c.683C>A	c.502C>T	c.831C>G	-
Predicted protein change	p.Lys297Glu	p.Tyr106*	p.Phe65Leu	p.Leu172Pro	p.Ser308Phe	p.Gly8Serfs*9	p.Met1?	p.Val331Ile	p.Tyr145Profs*76	p.Phe324Leufs*25	p.Ser213Phe	p.Tyr267Cys	p.Gly317Alafs*32	p.Gly266Val	p.Val258Met	p.Ser228*	p.Gln168*	p.Asn277Lys	-
Isf Tf (age-yr;mo)	nd	nml (8;11)	nml (3;4)	nml (2;6)	nd	abn (0;5), nml (3;2)	abn (0;7), nml (2;9)	abn (0;5-7), nml (5;0)	nml (8;10)	nml (12;8)	nml (10;5)	nd	mass spectrometry nml (1;0),(2;6)	abn (0;1), (5;0)	nml (8;0)	nd	nd	abn (2;0)	-
Dev. delay	S	S	S	S	M-S	Y	Y	Y	S	S	S	Y	S	S	S	nd	S	S	17 of 17 (100%)
ID	n/a	S	S	S	n/a	n/a	n/a	nd	S	S	S	S	n/a	nd	S	Y	S	nd	10 of 10 (100%)
Seizures	Y	Y	Y	Y	N	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	16 of 18 (89%)
Age seizure onset	6wk	5mo	6mo	6wk	n/a	nd	nd	nd	6 days	1mo	3mo	nd	2mo	3mo	nd	3mo	4.5mo	nd	-
Initial seizure type	Infantile spasms	Infantile spasms	Infantile spasms	Infantile spasms	n/a	nd	nd	nd	Tonic	Infantile spasms	Infantile spasms	nd	Partial motor seizures upper limbs	nd	nd	Infantile spasms	Infantile spasms	nd	-
Evolving seizure phenotype	Myoclonic and tonic + ongoing spasms with clusters	Drop attacks + tonic spasms	n/a	n/a	n/a	nd	nd	nd	Infantile spasms + tonic seizures	Tonic seizures, spasms, focal seizure	Tonic seizure, spasms	nd	Spasms	nd	nd	Spasms + tonic seizures	Myoclonic, tonic-clonic, tonic	nd	-

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Ng et al. (Patient 1)	Ng et al. (Patient 2)	Ng et al. (Patient 3)	Kodera et al. (Patient 1)	Kodera et al. (Patient 2)	Kodera et al. (Patient 3)	Bosch et al.	Kimizu et al.	Dorre et al.	Lopes et al.	EuroEPINOMICS et al (Patient 1)	EuroEPINOMICS et al (Patient 2)	Evers et al.	Total (reported)
Seizures ctrl	N	N	Y	Y	n/a	nd	nd	nd	N	N	N	nd	Y	Y	nd	N	N	nd	4 of 11 (36%)
Hypsarrhythmia	Y	Y	Y	Y	N	Y	Y	N	Y	Y	Y	nd	Y	Y	N	Y	Y	nd	13 of 16 (81%)
Dysmorphism	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	nd	16 of 17 (94%)
Hypotonia	N	N	Y	Y	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	N	Y	N	N	12 of 18 (67%)
Microcephaly	N	Y	N	N	N	N	Y	Y	nd	nd	nd	Y	N	Y	Y	nd	nd	N	6 of 13 (46%)
MRI abn.	TC, AV	DM	N	DM, TC	N	Small cerebellum	DM, TC	CA	TC, DM, CA	AV	CA, TC	DM	TC, CA, AV	CA, TC	CA, TC, periventricular heterotopia	N	DM, CA	CA	15 of 18 (83%)
Visual defect	CV	N	RA	N	EM	EM	RA	EM	N	RA	N	CV	N	'severe visual impairment'	nd	nd	EM	Cataract	11 of 16 (69%)
Skeletal abn.	SC	CV	JH, GV	N	N	N	SE	SE	N	N	HD	SC	nd	JH	nd	nd	SC	nd	9 of 14 (64%)

Table 2. Summary of patients with pathogenic SLC35A2 variants previously reported, including this study cohort.
Brain abnormalities: TC: thin corpus callosum, AV: abnormal ventricles, DM: delayed myelination, CA: cerebral/cerebellar atrophy,
Ophthalmic abnormalities: CV: cortical visual impairment, RA: refractive error, EM: abnormal eye movements, RA: retinal abnormality
Skeletal abnormalities: SC: scoliosis, CV: coxa valga, GV: genu valga, SE: shortened extremities, HD: hip dislocation, JH: joint hypermobility
Abbreviations: Inher. - inheritance, yr - year, mo - months, dn - de novo, mat - maternal, Y - yes, N - no, ACMG-AMP - American College of Medical Genetics and Genomics – Association for Molecular Pathology, Isf Tf - Isoelectric focusing of transferrins S - severe, M-S – moderate to severe, ctrl - controlled, nd - not documented, Dev - developmental, ID - intellectual disability, nml - normal, abn - abnormality, ophthal - ophthalmic

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Conflicts of interest

None to declare for all authors